REMARKS

Claim 1 has been amended to more closely define the panel of monoclonal antibodies used in the claimed method of screening. Claim 1 now specifies that the panel "binds to surface antigens on normal cells of the cervix in a pattern which represents normality" and basis for this amendment appears on page 3, lines 10 to 14 of the Application as filed.

Additionally, Claim 1 now specifies that the panel must include a monoclonal antibody which is specific for columnar cells. Page 13, lines 17 to 23 state:

> "The squamo-columnar junction is of clinical importance as it is the region where the majority of malignancies arise. For diagnostic validity, a cervical smear sample must include cells from this region. In order to ensure that this has been achieved, a smear must contain columnar as well as squamous epithelial cells".

In the examples, the antibody 2C7 reacts "specifically and solely with columnar epithelial cells" (page 37, lines 23-24). Also see page 38, lines 1-3 where it is stated that 2C7 "reacted specifically with columnar epithelial cells, and not with any other cell population in the cervix".

Further, Claim 1 now specifies that the panel must include a monoclonal antibody able to bind to squamous cells, and that the percentage binding of this antibody to squamous cells from a premalignant or neoplastic sample is reduced with respect to normal cervical squamous cells. Basis for this amendment is found on page 47, line 26 to page 48, line 5, and Tables 3 and 4 of the present specification. As is discussed in these passages, both HG3 and 9G5 demonstrate reduced binding to squamous cells in premalignant/neoplastic samples. In Table 4, patients 6 and 7,

are diagnosed with the conventional PAP test as being premalignant/neoplastic, but according to the screening method the binding of 9G5 and HG3 appears normal. This apparent anomaly is discussed on page 50, lines 3 to 9 when further investigations revealed that "visually these samples appeared similar to normal smears" and that "dyskaryotic cells were not apparent". In other words, the conventional PAP test had erroneously identified these samples as being premalignant/neoplastic (*i.e.*, a false positive result), whereas the methodology of the invention had correctly identified them as normal.

Claim 3 has also been amended to specify that binding of the monoclonal antibodies to cells of a premalignant or neoplastic sample is reduced with respect to normal cervical squamous cells. Basis for this amendment is found on page 47, line 26 to page 48, line 5, and in Tables 3 and 4.

New Claims 10 and 12 have been added to specifically refer to the panel including a monoclonal antibody having an antigen binding domain obtainable from the hybridoma deposited under No. ECACC 95020716.

New Claim 11 refers to surface antigens which are not cytokeratins. Basis for this amendment may be found at page 27, lines 6 to 25 which confirms that all of the antibodies used in the examples do not bind to cytokeratins.

Objections were raised under 35 USC § 102(b) and 35 USC § 103 to Claims 1, 2 and/or 8 only. Claim 8 has now been deleted.

Claims 1 and 2 were rejected under 35 USC 102(b) as being anticipated by Porta et al.

The Examiner rejected Applicant's previous arguments and stated:

"However, Porta et al. teach that monoclonal antibodies have been successfully used by others to screen for neoplasia of the cervix by staining cells in a cervical smear sample. While Porta et al. do teach that Jha et al. reported that one particular panel of monoclonal antibodies were not successful in differentiating neoplastic and normal cells, they also teach that other researchers have successfully used monoclonal antibodies for differentiation of neoplastic and normal cells in cervical smear samples" (Page 2, last paragraph of the Office Action).

Applicant has studied Porta et al. carefully, but can find no reference to "other researchers" successfully using monoclonal antibodies to differentiate between neoplastic and normal cells in cervical smear samples as stated by the Examiner. Porta et al. is in fact predominately concerned with diagnosis of carcinoma in the uterine cervix, that is establishing the presence of ~~a frank tumor~~. By contrast, the claims of the current Application are concerned with screening for a premalignant or neoplastic disease state, in other words locating cellular changes which precede the development of the tumor.

Porta et al. does not teach that it is possible to recognize a pattern of surface antigens on the cells of the cervix which represents normality, such that a deviation from the determined pattern of normality can be perceived and relates to a premalignant or neoplastic disease state. Porta et al. is by contrast only concerned with the recognition of tumors by specific monoclonal antibody binding to the tumor tissue. In the present invention, binding to a tumor does not occur; screening is of normal or premalignant/neoplastic cells.

Porta et al. does not teach that neoplastic or premalignant cells on the cervix can be distinguished by means of monoclonal antibody binding, since Porta et al. is only concerned with tumor

diagnosis. In carcinoma much more significant cellular changes have occurred relative to normal tissue than is the case with tissue exhibiting premalignant/neoplastic characteristics, and hence diagnosis of carcinoma does not support a reasonable expectation of success in screening for premalignant/neoplastic cellular changes.

As stated the above, the Examiner's reference to the "other researchers" made in the Office Action is not understood. The Examiner is requested to clarify this objection by clearly stating which researchers are believed to be relevant.

Claims 1 and 2 were rejected under 35 USC 102(b) as being anticipated by Smedts et al.

Smedts et al. reports the expression of certain keratins in normal cellular epithelium, CIN and cervical carcinoma. Smedts et al. states that the "tissue specimens used in this study were taken from diathermy loop excision specimens or cervical cone biopsies" (Page 404, column 2). Thus, the tissue specimens used by Smedts et al. represent an intact section of cervical tissue in which cellular organization and hierarchy is maintained. The results presented in Figure 1 of Smedts et al. confirms that the cellular organization was intact since the results are presented for each cell type present in the tissue specimen, even where binding to multiple cell types occurs. The types of tissue present in such samples include the deeper layers of squamous cells.

By contrast, in a cervical smear a different proportion of cells are sampled, and the cellular organization and hierarchy is lost since the process of taking a smear sample does not allow presentation of the tissue structure in any way. Moreover in a smear the more superficial layers of squamous cells are much more highly represented when the cervix is sampled; the deeper

squamous layers are either poorly represented or not present at all, as shown in Table 3 on Page 54. Claims 1 and 2 specify that the method concerns a cervical smear sample. This differs markedly from the cone biopsy and diathermy loop biopsy samples of Smedts et al. The Examiner is referred to page 13, line 5 through to page 15, line 2 of the present Application for a description of the cell types of the cervix. In particular, the Examiner is referred to page 13, lines 16-22 which states:-

> "The squamo-columnar junction is of clinical importance as it is the region where the majority of malignancies arise. For diagnostic validity, a cervical smear sample must include cells from this region. In order to ensure that this has been achieved, a smear must contain columnar as well as squamous epithelial cells".

In the Smedts et al. document only keratin 7 reliably binds to columnar cells in all women. The remaining keratins are not reliably expressed in all women as is demonstrated by the hatched shading in Fig. 1 of Smedts et al. However keratin 7 is also expressed in squamous cells in CIN, giving rise to a false positive result.

As amended, Claim 1 states that the panel of monoclonal antibodies must include at least one antibody which is specific for columnar cells. Smedts et al. lacks such a requirement.

Claims 1 and 2 were rejected under 35 USC 103(a) as obvious over Smedts et al. The Examiner argues that the monoclonal antibodies of Smedts et al. would react to the same antigens even if a cervical smear sample is tested. However, as explained above, for a cervical smear sample to be diagnostically adequate columnar cells must be present. The methodology of Smedts et al. does not distinguish between a normal sample containing columnar cells and a CIN sample - since both express keratin 7.

Smedts et al. fail to recognize the importance of sampling columnar cells to establish the diagnostic validity of a cervical smear sample, since Smedts et al. are concerned only with diathermy loop excisions and cone biopsies.

An objection under 35 USC 102(b), alternatively under 35 USC 103(a) in view of Porta et al. or Smedts et al. was raised to Claim 8. Claim 8 has been deleted.

The Examiner rejected Claims 1-4 under 35 USC § 112, first paragraph. The Examiner referred back to the Advisory Action and the previous Office Action, mailed November 7, 2001.

In item No. 7, page 6, 2nd paragraph of the November 7, 2001 Office Action the Examiner states:

> "The portions of the disclosure referenced at pages 3 and 4 are general disclosures regarding the use of a panel of five monoclonal antibodies for detection of marker antigens on cervical cells and a general teaching that the pattern of binding of these antibodies differs in normal and abnormal cell samples. The data to support this disclosure is presented in Example 5, Tables 3 and 4. Firstly, it is somewhat difficult to interpret the data presented in Tables 3 and 4 because the meaning of the "ve" is not defined in either the disclosure or the footnotes. Secondly, Tables 3 and 4 disclose staining patterns of 10 normal samples and 10 premalignant specimens with a panel of 5 monoclonal antibodies, not 2 or more, as is claimed. Thirdly, it is not clear that the staining pattern of the premalignant specimens is significantly different from that of normal specimens in a significant number of specimens so as to be diagnostic of disease. A number of normal and premalignant specimens appear to be reactive with 9G5 AND HG3 and

unreactive with two or more of the remaining 3 MAB. There does not appear to be any data whatsoever regarding differentiation of neoplastic specimens from normal specimens. Lastly, applicant's claims are drawn to a method of screening for a premalignant or neoplastic disease. Methods of clinical diagnosis of premalignant and neoplastic disease are clearly envisioned and encompassed within applicant's claimed method".

Five monoclonal antibodies are indeed identified on page 16 and their binding is discussed in the examples.

The data is given for all five antibodies for simplicity of presentation. Thus, for example, the antibody 9G5 and the antibody HG3 show similar characteristic antibody reactivity profiles, being negative for basal cells, parabasal cells and columnar cells and positive for intermediate cells and superficial cells. At the time of filing the application, Applicant included all relevant data of all antibodies investigated. Had Applicant only investigated three antibodies at that time, a smaller panel would have been presented. Conversely, had Applicant had data on twenty antibodies at the time of filing these would also have been presented in Tables 3 and 4. The requirement of the panel has now been more closely defined as requiring a monoclonal antibody specific to columnar cells and a monoclonal antibody specific to squamous cells. The data presented supports this requirement; there is no particular significance or "magic" to five monoclonal antibodies as appears to be believed by the Examiner.

The Examiner states that the data presented in Example 5, Table 3 (page 54) and Table 4 (page 55) is difficult to interpret as the meaning of "ve" is not given. It is submitted that "-ve" is generally well known and recognized as meaning negative and "+ve" as meaning positive. Hence in Tables 3 and 4 those samples marked

"-ve" did not show any binding to the antibody in question. In Tables 3 and 4 the "+ve" result indicated instances where clumps of stained cells were observed, as explained by the footnote designated "$". A typographical error has occurred in Table 3, in which the "-ve" result for BC4 binding to Specimen 5 has been denoted as "ve". It is nonetheless clear that "-ve" is intended as no footnote reference ($) is superscripted.

The Examiner notes that:

> "it is not clear that the staining pattern for the premalignant specimens is significantly different from that of normal specimens in a significant number of specimens so as to be diagnostic of disease. A number of normal and premalignant specimens appear to be reactive with 9G5 and HG3 and unreactive with two or more of the remaining 3 MAB."

This comment from the Examiner illustrates the issue in point; namely that 9G5 and HG3, being reactive for squamous cells, are actually providing diagnosis, the remaining cells merely provide information on the presence of the different cell types within the smear sample. (The smear itself lacking the organization of cells as present on the cervix). Indeed, the data presented in Table 3 shows that the smear samples tested for patients 1, 2, 4, 5, 6 and 10 were inadequate for a proper diagnosis to be made since these samples were not positive for the 2C7 antibody specific for columnar cells. Likewise in Table 4 the smear samples tested for patients 5, 6 and 8 were inadequate as no columnar cells were present, and these patients should have been retested. These results demonstrate one of the important features of the present invention which ensures diagnosis is only made on the basis of a competent sample. This provides a marked improvement over the currently available (PAP) testing which fails to recognize the inadequacy of these samples.

Finally, the Examiner is concerned that Table 4 apparently only tests "pre-malignant" samples whereas the Claims are drawn to a method of screening for "a premalignant or neoplastic disease". Enclosed herewith is a Declaration under 37 C.F.R. §1.132 from Dr Christopher Holmes, a co-inventor of this Application attesting that these two terms were used interchangeably and were deemed to be equivalent to each other at the time of filing the Application.

Claims 1, 2 and 4 were rejected under 35 USC 112, first paragraph, on the basis that the phrase "having different specificities and raised against antigens present on normal cervical tissue" was deemed not to have support in the Application as filed. The objected phrase has been deleted.

Conclusion

Based on the foregoing, all claims are believed in condition for allowance. An early and favorable action toward that end is earnestly solicited.

Respectfully submitted,
ROBERT JAMES MASON *et al.*

BY _____

DANIEL A. MONACO
Registration No. 30,480
DRINKER, BIDDLE & REATH, LLP.
One Logan Square
18th and Cherry Streets
Philadelphia, PA 19103
(215) 988-3304 ph.
(215) 988-2757 fax
Attorney for Applicants

MARKED UP VERSION OF THE CLAIMS

1. (AMENDED FOUR TIMES) A method of screening for a premalignant or neoplastic disease state in a cervical smear sample containing cells of the cervix, the method comprising contacting said sample with a panel of two or more monoclonal antibodies [having different specificities and raised against antigens present on normal cervical tissue], wherein said panel binds to surface antigens on normal cells of the cervix in a pattern which represents normality, said panel including at least one monoclonal antibody specific for columnar cells and at least one monoclonal antibody specific for squamous cells, determining binding of said monoclonal antibodies to said sample and comparing the binding with a pattern of binding of said monoclonal antibodies to a normal cervical cell sample, wherein the percentage binding of the at least one monoclonal antibody specific for squamous cells to premalignant or neoplastic cells is decreased with respect to [said monoclonal antibodies detect cellular markers which differ between] normal [and premalignant or neoplastic] cells.

3. (AMENDED THREE TIMES) A method of determining a premalignant or neoplastic disease state in a cervical smear sample containing cells of the cervix, the method comprising contacting a panel of two [one] or more monoclonal antibodies with said sample, determining binding of said monoclonal antibodies to said sample and comparing the binding with a pattern of binding of said monoclonal antibodies to a normal cervical cell sample, wherein the percentage binding of the two or more monoclonal antibodies to premalignant or neoplastic cells is decreased with respect to the percentage binding of said monoclonal antibodies [detect cellular marks which differ between] to normal [and premalignant or neoplastic] cells, and wherein the panel includes one or

more monoclonal antibodies [comprise] comprising one or more polypeptides each comprising an antigen binding domain obtained from a hybridoma selected from those deposited at the European Collection of Animal Cell Cultures (ECACC), under the accession numbers ECACC 95020718, ECACC 95020716, ECACC 95020720, ECACC 95020717 and ECACC 95020719.

4. (AMENDED FOUR TIMES) A method according to Claim 1 wherein one or more of the monoclonal antibodies comprise [substances] a polypeptide able to bind to an antigen which can be bound by one or more antibodies obtained from a hybridoma selected from those deposited at the European Collection of Animal Cell Cultures (ECACC), under the accession numbers ECACC 95020718, ECACC 95020716, ECACC 95020720, ECACC 95020717 and ECACC 95020719.